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HUMAN OSTEOCLAST MEDIATED DEGRADATION OF ARTICULAR CARTILAGE - AN IN VITRO MODEL FOR RHEUMATOID ARTHRITIS

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Purpose: Rheumatoid arthritis (RA) is characterized by chronic inflammation of the synovium, which is followed by degradation of the articular cartilage and the subchondral bone. Monocytes present in the synovium differentiate into osteoclasts under the inflammatory conditions. The osteoclasts express high levels of proteolytic enzymes such as cathepsins and matrix metalloproteinases (MMP), which mediate a pathological degradation of both cartilage and bone. The objective of this study was to investigate osteoclast mediated articular cartilage degradation, evaluated by the collagen type II degradation marker CTX-II and glycosaminoglycan (GAG) release, as a model for RA.
Research Methods: The study is based on in vitro investigations using two experimental approaches. 1) We investigated whether human osteoclasts (HOCs) generated from CD14+ monocytes were able to degrade bovine articular cartilage. The degradation was measured as the release of C-terminal telopeptides of collagen type II (CTX-II) and GAGs, in the presence or absence of MMP or cysteine proteinase inhibitors. In addition, we used immunocytochemistry to demonstrate localization of osteoclasts (TRACP) and sites of CTX-II release in the explants. Human osteoclasts on cortical bovine bone slices were used as controls for the effect of the inhibitors. 2) The effect of recombinant MMPs and Cathepsin K on cartilage degradation was investigated using metabolically inactivated (snap frozen) cartilage explants.
Results: Release of CTX-II and GAGs from metabolically inactive explants was induced by HOCs. In addition, the inhibitor, GM6001, could suppress the CTX-II and GAG release, whereas the cystein proteinase inhibitor, E64, had no effect of the CTX-II release, but reduced the GAG release by 50%. In contrast, resorption of cortical bone by human osteoclasts was reduced 90% by E64 and unaffected by GM6001 as expected. Immunohistochemistry revealed that sites of CTX-II release co-localized with the presence of TRACP-positive osteoclasts.
In correlation with the osteoclast mediated cartilage degradation, CTX-II release from metabolically inactive explants induced by recombinant MMPs (MMP-9 and 13) was 1000-fold higher than that induced by Cathepsin K ($p<0.001$).
Conclusions: These results demonstrate that HOCs are capable of degrading bovine articular cartilage suggesting that this system can be used as an in vitro model for RA. Moreover, the osteoclasts generate the CTX-II fragment in a MMP dependent manner, whereas the cystein proteinases do not generate CTX-II fragments, although the cystein proteinases are partially involved in GAG release.

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HIGH-LEVEL EXPRESSION OF DISCOIDIN DOMAIN RECEPTOR 2 (DDR2) IN HUMAN ARTHRITIC CARTILAGES AND KNEE ARTICULAR CARTILAGE OF A SURGICALLY INDUCED MOUSE OA MODEL

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In previous studies, we found that the protein expression of discoidin domain receptor 2 (Ddr2, a cell membrane tyrosine kinase), which binds preferentially to type II collagen, was increased in knee articular cartilages of type IX collagen-deficient and type XI collagen haploinsufficient mice. These two mutant mouse strains exhibit age-dependent OA-like changes in the knee and temporomandibular (TM) joints. To determine whether or not the expression of DDR2 was increased in human OA, we collected seven arthritic hip joints (surgical wastes from the hip replacement) from patients of ages between 65 to 85 years old and four hip joints (surgical wastes from the hip replacement due to the bone fracture) from individuals without arthritic symptoms, aged between 60 to 80 years old. Multiple 0.5x0.5 cm areas of full-thickness articular cartilage were excised from each joint, fixed in 4% paraformaldehyde, and embedded in paraffin. Serial sections of 8-um thickness were cut and very fifth section was collected for routine histology. The adjacent paraffin sections were used for staining of DDR2, MMP-13 and degraded type II collagen. We also collected six knee joints from mice with surgically induced OA and six knee joints from the control mice. To induce OA surgically, the intercondylar region was exposed to provid visualization of the meniscotibial ligament of the medial meniscus. The medial meniscotibial ligament was transected, resulting in destabilization of the medial meniscus and increased mechanical stress on the articular cartilage. Control joints included no surgery and sham surgery in which the ligament was visualized but not transected. For analyses, the mice were sacrificed at 2 and 4 weeks post-operatively and the knee joints were fixed in 4% paraformaldehyde, decalcified, and embedded in paraffin. A serial frontal section (5-um thickness) was cut through the entire joint. Three successive paraffin sections from three different locations, anterior, medial and posterior, were collected for histology. Successive paraffin sections next to the ones used for the histology were used for staining of Ddr2, Mmp-13 and degraded type II collagen. Results from histology examination showed proteoglycan depletion and loss of articular cartilage in human arthritic hip joints and the articular cartilage of knee joints of surgical OA mice. Results from immunostaining indicated that the expressions of DDR2 and MMP-13 were increased in both arthritic cartilage samples, associated with the increased amount of degraded type II collagen. These data suggest that the expression of DDR2 and MMP-13 is increased both in human OA and in the surgically induced mouse OA model.